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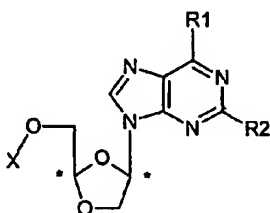
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<b>(21) International Application Number:</b> PCT/CA00/00212 <b>(22) International Filing Date:</b> 01 March 2000 (01.03.2000) <b>(30) Priority Data:</b> 60/122,480 01 March 1999 (01.03.1999) US <b>(60) Parent Application or Grant</b> BIOCHEM PHARMA INC. [/]; (). RANDO, Robert [/]; (). GU, Zhengxian [/]; (). RANDO, Robert [/]; (). GU, Zhengxian [/]; (). MURPHY, Kevin, P. ; ().	<b>Published</b>	
<b>(54) Title: PHARMACEUTICAL COMBINATION OF ANTIVIRAL AGENTS</b> <b>(54) Titre: COMBINAISON PHARMACEUTIQUE D'AGENTS ANTIVIRAUX</b>  <b>(57) Abstract</b> <p>In accordance with the present invention there is provided a pharmaceutical combination useful for the treatment of viral infections comprising a at least one antiviral active compound of formula (1), and at least one further therapeutic agent chosen from nucleoside analogues; NNRTIs; and protease inhibitors.</p> <b>(57) Abrégé</b> <p>L'invention concerne une combinaison pharmaceutique utile pour le traitement d'infections virales comprenant au moins un principe actif antiviral de formule (1) et au moins un agent thérapeutique sélectionné dans les analogues de nucléoside; NNRTI; et des inhibiteurs de la protéase.</p>		

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(54) Title: PHARMACEUTICAL COMBINATION OF ANTIVIRAL AGENTS			
<div style="text-align: center;"> (1)</div>			
(57) Abstract			
In accordance with the present invention there is provided a pharmaceutical combination useful for the treatment of viral infections comprising a at least one antiviral active compound of formula (1), and at least one further therapeutic agent chosen from nucleoside analogues; NNRTIs; and protease inhibitors.			

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**Description**

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## FIELD OF THE INVENTION

## BACKGROUND OF THE INVENTION

Although mortality rates from AIDS are dropping due to new  
30 therapies, AIDS remains the second leading cause of death  
in adults between the ages of 29 and 40. Combination anti-

5 HIV therapy is now the standard of care for people with  
HIV. There are now 11 anti-HIV drugs available by  
prescription. These anti-HIV drugs fall into three  
10 categories: nucleosides analogs, which include zidovudine,  
5 didanosine, zalcitabine, stavudine and lamivudine;  
protease inhibitors which include indinavir, nelfinavir,  
saquinavir and ritonavir and non-nucleoside reverse  
15 transcriptase inhibitors (NNRTI) which include nevirapine,  
delavirdine and efavirenz. Compared to HIV, there is  
10 presently only two licensed therapy for chronic hepatitis  
B virus infection which are interferon and lamivudine.  
20 Other drugs are currently under clinical trials including  
lamivudine, famciclovir, lobucavir and adefovir. But many  
studies have shown that most patients relapse after  
25 completion of therapy and develop resistance to the drugs.

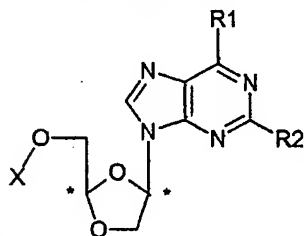
Development of resistance has recently become a major  
concern in the treatment of HIV and HBV infections.  
30 Resistance usually occurs when the drugs being used are  
20 not potent enough to completely stop virus replication. If  
the virus can reproduce at all in the presence of drugs,  
it has the opportunity to make changes in its structure,  
35 called mutations, until it finds one that allows it to  
reproduce in spite of the drugs. Once a mutation occurs,  
25 it then grows unchecked and soon is the dominant strain of  
the virus in the individual. The drug becomes  
40 progressively weaker against the new strain. There is also  
increasing concern about cross-resistance. Cross-  
resistance occurs when mutations causing resistance to one  
45 30 drug also cause resistance to another. Several studies  
have proven that combining two drugs delays the

development of resistance to one or both drugs compared to when either drug is used alone. Other studies suggest that three-drug combinations extend this benefit even further. As a result, many people believe that the best way of preventing, or at least delaying resistance is to use multi-drug combination therapies. But as the number of drugs increases, so does the risk of drug interactions and toxicity.

(-)- $\beta$ -D -2,6-diaminopurine dioxolane (DAPD) and (-)- $\beta$ -D -1,3-dioxolane guanine (DXG) have been reported to be highly efficacious against HIV-1 in various cell systems, have minimal cross resistance with lamivudine, and low cellular toxicity. Combinations of DAPD and DXG with other therapeutic agents which exhibit potent therapeutic activity against HIV and HBV would greatly aid in the development of new combination therapy against HIV and HBV.

#### SUMMARY OF THE INVENTION

In one aspect, the present invention provides a novel pharmaceutical combination useful for the treatment of viral infections comprising a at least one antiviral active compound of formula (I) :



(I)

and pharmaceutically acceptable salts thereof,

wherein:

where R<sub>1</sub> is chosen from O and the formula -NR<sub>3</sub>R<sub>4</sub> wherein:

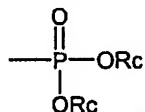
R<sub>3</sub> is a saturated or unsaturated C<sub>3-8</sub> carbocyclic ring optionally substituted with COOH, CONH<sub>2</sub>, OH, SH, NH<sub>2</sub>, NO<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, COR<sub>a</sub> wherein R<sub>a</sub> is a C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl and COOR<sub>b</sub> wherein R<sub>b</sub> is a C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl;

R<sub>4</sub> is H or a C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl;

R<sub>3</sub>R<sub>4</sub> can also be connected to the nitrogen atom to form a saturated or unsaturated C<sub>3-8</sub> heterocyclic ring optionally substituted with COOH, CONH<sub>2</sub>, OH, SH, NH<sub>2</sub>, NO<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, COR<sub>a</sub> wherein R<sub>a</sub> is a C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl and COOR<sub>b</sub> wherein R<sub>b</sub> is a C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl;

R<sub>2</sub> is chosen from H, halogen and NH<sub>2</sub>.

X is chosen from H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>6-10</sub> aryl and



wherein each R<sub>c</sub> is independently chosen from H, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl and an hydroxy protecting group;

wherein said nucleoside is present in the form of the (-) enantiomer, (+) enantiomer and mixtures thereof, including racemic mixtures;



5 and at least one further therapeutic agent chosen from  
nucleoside analogues; NNRTIs (non nucleoside reverse  
transcriptase inhibitors); and protease inhibitors

10 5 The pharmaceutical combinations of the present invention  
are useful in therapy, in particular as antivirals.

15 In another aspect, there is provided a method of treating  
viral infections in a subject in need of such treatment  
10 comprising administering to the subject a therapeutically  
effective amount of a compound or composition of the  
20 invention.

25 In another aspect, there is provided a pharmaceutical  
15 formulation comprising the compound of the invention in  
combination with a pharmaceutically acceptable carrier or  
excipient.

30 In another aspect of the invention is the use of a  
20 compound according to formula I, for the manufacture of a  
medicament for the treatment of viral infections.

35 BRIEF DESCRIPTION OF THE FIGURES

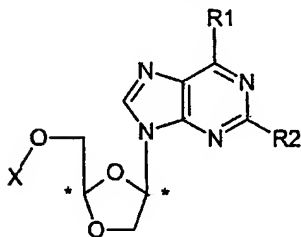
Figure 1 represents the dose response curve of inhibition  
25 of HIV-1 replication. MT-2 cells were infected with HIV-  
1IIIB at an MOI of 0.005. The infected cells were cultured  
40 in the presence of various concentrations of antiviral  
compound as shown in this Fig. Viral susceptibility to the  
compounds was assayed by measurement of HIV-1 RT activity  
45 30 in the culture supernatants as described in Methods. Data

are expressed as means  $\pm$  standard deviations for at least five separated experiments, each performed in duplicate. Figure 2 represents the comparison of chain-termination effect of DXG-TP with other dideoxynucleotide triphosphates and NNRTI on reverse transcription. The bands at the top of the gel were full-length cDNA products in this assay. The solid arrows show examples of chain termination bands generated by each of the dideoxynucleotide inhibitors.

#### DETAILED DESCRIPTION OF THE INVENTION

In one embodiment, compounds of the present invention comprise those wherein the following embodiments are present, either independently or in combination.

In one aspect, the present invention provides a novel pharmaceutical combination useful for the treatment of viral infections comprising a at least one antiviral active compound of formula (1) :



and pharmaceutically acceptable salts thereof,  
wherein:

R1 is chosen from O and the formula  $-NR_2R_3$ , wherein:

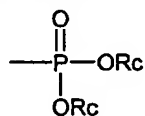
5  $R_1$  is a saturated or unsaturated  $C_{3-8}$  carbocyclic ring optionally substituted with  $COOH$ ,  $CONH_2$ ,  $OH$ ,  $SH$ ,  $NH_2$ ,  $NO_2$ ,  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl, halogen,  $COR_a$  wherein  $R_a$  is a  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl and  $COOR_b$  wherein  $R_b$  is a  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl;

$R_4$  is  $H$  or a  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl;

15  $R_3R_4$  can also be connected to the nitrogen atom to form a saturated or unsaturated  $C_{3-8}$  heterocyclic ring optionally substituted with  $COOH$ ,  $CONH_2$ ,  $OH$ ,  $SH$ ,  $NH_2$ ,  $NO_2$ ,  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl, halogen,  $COR_a$  wherein  $R_a$  is a  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl and  $COOR_b$  wherein  $R_b$  is a  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl;

25  $R_2$  is chosen from  $H$ , halogen and  $NH_2$ .

$X$  is chosen from  $H$ , monophosphate, diphosphate, triphosphate, carbonyl substituted with a  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl,  $C_{6-10}$  aryl and



35 20 wherein each  $Rc$  is independently chosen from  $H$ ,  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl and an hydroxy protecting group;

wherein said nucleoside is present in the form of the (-) enantiomer, (+) enantiomer and mixtures thereof, including racemic mixtures;

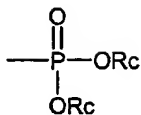
40 25 and at least one further therapeutic agent chosen from nucleoside analogues; NNRTIs; and protease inhibitors.

45 In one embodiment,  $X$  is chosen from  $H$ , monophosphate, diphosphate and triphosphate.

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In one embodiment, X is H.

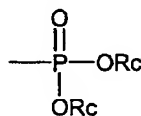
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Alternatively X is wherein each Rc are independently chosen from phosphate, diphosphate H, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl and an hydroxy protecting group chosen from S-acylthioethyl ester, acyloxymethyl ester and alkyl methyl carbonate.

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In one embodiment, X is wherein each Rc are independently an hydroxy protecting group chosen from acetyl-2-thioethyl ester, pivaloyloxymethyl ester and isopropylloxycarbonyloxymethyl ester.

30

In one embodiment, R<sub>1</sub> is represented by NH<sub>2</sub> or O

In a further embodiment, R<sub>2</sub> is H or methyl.

35

In a further embodiment, R<sub>3</sub> is H.

40

In a further embodiment R<sub>4</sub> is chosen from H, COOH, CONH<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl and COOR<sub>5</sub> wherein R<sub>5</sub> is a C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl.

45

In a further embodiment R<sub>4</sub> is H, COOH, or C<sub>1-6</sub> alkyl.

In a further embodiment R<sub>4</sub> is H, COOH, methyl or ethyl.

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5 In a further embodiment  $R_4$  is methyl or ethyl.

In an alternative embodiment,  $R_4$  is COOH.

10 5 In a further embodiment  $R_4$  is H.

In a further embodiment,  $R_3$  is H or methyl and  $R_4$  is H.

15 In a further embodiment  $R_4$  and  $R_3$  are H.

10 In one embodiment,  $R_2$  is chosen from H, halogen and  $NH_2$ .

20 In a further embodiment,  $R_2$  is Cl or  $NH_2$ .

25 15 In one embodiment,  $R_2$  is  $NH_2$ .

In one embodiment, the pharmaceutical combinations of this invention may contain at least one other antiviral agent chosen from 3TC (lamivudine), AZT (zidovudine), FTC (5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine), d4T (2',3'-dideoxy-2',3'-didehydrothymidine, stavudine and Zerit), nevirapine, DMP-226, nelfinavir, delavirdine, 9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine, 2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine, MKC-442, 1592U89 (abacavir), 141W94, MK-639, BMS-234475, PNU-140690, ABT-378, DMP-450, Indinavir, saquinavir, ritonavir, efavirenz (sustiva), TIBO, HEPT, BHAP,  $\alpha$ -APA, TSAO, calanolides, L-697,661, 2',3'-dideoxycytidine (ddC or zalcitabine), 2',3'-dideoxyadenosine, 2',3'-dideoxyinosine (ddI or didanosine), 3'-deoxythymidine and 2',3'-dideoxy-2',3'-

5 didehydrocytidine and ribavirin; acyclic nucleosides such  
as acyclovir, ganciclovir, interferons such as alpha-,  
beta-and gamma-interferon; glucuronation inhibitors such  
10 as probenecid; nucleoside transport inhibitors such as  
5 dipyridamole; immunomodulators such as interleukin II  
(IL2) and granulocyte macrophage colony stimulating factor  
(GM-CSF), erythropoietin, ampligen, thymomodulin,  
15 thymopentin, foscarnet, glycosylation inhibitors such as  
2-deoxy-D-glucose, castanospermine, 1-deoxynojirimycin;  
10 and inhibitors of HIV binding to CD4 receptors such as  
soluble CD4, CD4 fragments, CD4-hybrid molecules and  
20 inhibitors of the HIV aspartyl protease such as L-735,524.

In one embodiment, the pharmaceutical combinations of the  
25 present invention may contain at least one other antiviral  
agent chosen from zidovudine, didanosine, zalcitabine,  
stavudine, lamivudine, nevirapine, delavirdine, efavirenz,  
indinavir, nelfinavir, saquinavir and ritonavir.

30 In one embodiment, the pharmaceutical combinations of the  
present invention may contain at least one other antiviral  
agent chosen from chosen from zidovudine, lamivudine and  
35 nevirapine.

40 In one embodiment, the compounds of the invention are  
employed together with zidovudine, stavudine, or  
lamivudine.

45 In one embodiment, the compounds of the invention may be  
30 employed together with zidovudine.

5 In one embodiment, the compounds of the invention may be employed together with stavudine.

10 In one embodiment, the compounds of the invention may be employed together with lamivudine.

15 In one embodiment, the compounds of the invention may be employed together with nevirapine.

20 In one embodiment, the compounds of the invention may be employed together efavirenz.

25 The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

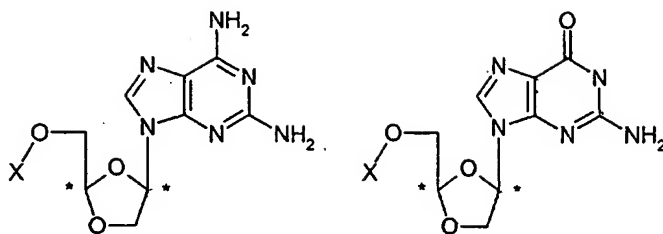
30 The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

35 In one embodiment, the pharmaceutical combination of the present invention include a compound of formula (Ia) and (Ib):

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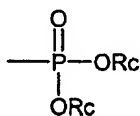


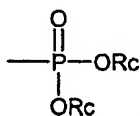
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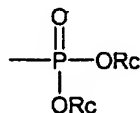
(Ib)

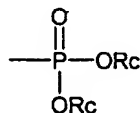
In one embodiment, X is chosen from H, monophosphate, diphosphate and triphosphate.

X is most preferably H.



Alternatively X is  wherein each Rc are independently chosen from H, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl and an hydroxy protecting group chosen from S-acylthioethyl ester, acyloxymethyl ester and alkyl methyl carbonate.



In one embodiment, X is  wherein each Rc are independently an hydroxy protecting group chosen from acetyl-2-thioethyl ester, pivaloyloxymethyl ester and isopropylloxycarbonyloxymethyl ester.

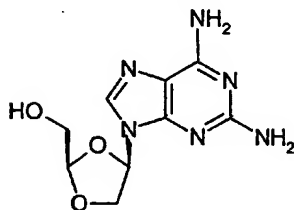
It will be appreciated by those skilled in the art that the compounds of formula (I) , (Ia) and (Ib) contain at



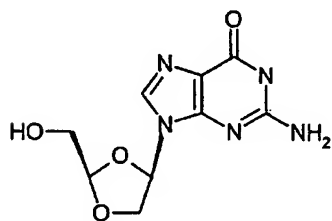
least two chiral centres which are marked by an asterisk (\*) on the general formula (I) and (Ia). The compounds of formula (I) and (Ia) thus exist in the form of two different optical isomers (i.e. (+) or (-) enantiomers or  $\beta$ -L and  $\beta$ -D). All such enantiomers and mixtures thereof including racemic mixtures are included within the scope of the invention. The single optical isomer or enantiomer can be obtained by method well known in the art, such as chiral HPLC, enzymatic resolution and chiral auxiliary.

In one embodiment, the pharmaceutical combination of the present invention include the one of the following compounds:

Compound A (-)- $\beta$ -D-2,6-diaminopurine-1,3-dioxolane (DAPD)



Compound B (-)- $\beta$ -D-1,3-dioxolane guanine (DXG)



In one embodiment, the compounds formula (I) (Ia) and (Ib) present in the pharmaceutical combination of the present

invention are provided in the form of a single enantiomer at least 95%, more preferably at least 97% and most preferably at least 99% free of the corresponding enantiomer.

In one embodiment, the compounds formula (1), (1a) and (1b) present in the pharmaceutical combination of the present invention are in the form of the (+) enantiomer at least 95% free of the corresponding (-) enantiomer.

In one embodiment, the compounds formula (1), (1a) and (1b) present in the pharmaceutical combination of the present invention are in the form of the (+) enantiomer at least 97% free of the corresponding (-) enantiomer.

In one embodiment, the compounds formula (1), (1a) and (1b) present in the pharmaceutical combination of the present invention are in the form of the (+) enantiomer at least 99% free of the corresponding (-) enantiomer.

In a further embodiment, the compounds formula (1), (1a) and (1b) present in the pharmaceutical combination of the present invention are in the form of the (-) enantiomer at least 95% free of the corresponding (+) enantiomer.

In one embodiment, the compounds formula (1), (1a) and (1b) present in the pharmaceutical combination of the present invention are in the form of the (-) enantiomer at least 97% free of the corresponding (+) enantiomer.

5 In one embodiment, the compounds formula (1), (1a) and (1b)  
present in the pharmaceutical combination of the present  
invention are in the form of the (-) enantiomer at least  
99% free of the corresponding (+) enantiomer.

10 5 In one embodiment, the compound of formula (1), (1a) and  
(1b) present in the pharmaceutical combination of the  
present invention is chosen from Compound A and Compound B

15 10 In one embodiment, the compound of formula (1), (1a) and  
(1b) present in the pharmaceutical combination of the  
present invention is Compound A

20 In one embodiment, the pharmaceutical combination of the  
present invention comprises at least one therapeutic agent  
is chosen from Compound A and Compound B and at least one  
additional therapeutic agent is chosen from zidovudine,  
25 didanosine, zalcitabine, stavudine, lamivudine,  
nevirapine, delavirdine, efavirenz, indinavir, nelfinavir,  
30 saquinavir and ritonavir.

35 In one embodiment, the pharmaceutical combination of the  
present invention is a synergistic combination of  
therapeutic agents comprising Compound A or Compound B and  
25 at least one additional therapeutic agent chosen from  
40 zidovudine, lamivudine and nevirapine.

45 There is also provided a pharmaceutically acceptable salts  
of the compounds formula (1), (1a) and (1b) present in the  
30 pharmaceutical combination of the present invention. By  
the term pharmaceutically acceptable salts of compounds of

5 general formula (1), (1a) and (1b) are meant those derived  
from pharmaceutically acceptable inorganic and organic  
acids and bases. Examples of suitable acids include  
10 hydrochloric, hydrobromic, sulphuric, nitric, perchloric,  
5 fumaric, maleic, phosphoric, glycollic, lactic, salicylic,  
succinic, toleune-p-sulphonic, tartaric, acetic, citric,  
methanesulphonic, formic, benzoic, malonic,  
15 naphthalene-2-sulphonic and benzenesulphonic acids. Other  
acids such as oxalic, while not in themselves  
10 pharmaceutically acceptable, may be useful as  
intermediates in obtaining the compounds of the invention  
20 and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal  
25 (e.g. sodium), alkaline earth metal (e.g. magnesium),  
ammonium and  $\text{NR}_4^+$  (where R is  $\text{C}_{1-4}$  alkyl) salts.

References hereinafter to the pharmaceutical combination  
30 according to the invention includes compounds of the  
20 general formula (1), (1a) and (1b) and there  
pharmaceutically acceptable salts.

35 By the term "heterocyclic ring " is meant a substituted  
(e.g. by a  $\text{C}_{1-6}$  alkyl, halogen, amino, or  $\text{NO}_2$ ), or  
25 unsubstituted, saturated or unsaturated,  $\text{C}_{1-6}$  cycloalkyl,  
40 wherein said cycloalkyl is interrupted by at least one  
heteroatom, e.g. oxygen, sulfur or nitrogen. Example of  
heterocyclic rings include but are not limited to epoxide;  
45 furane; oxathiolane; dithiolane; dioxolane; pyrrole;  
30 pyrrolidine; imidazole; pyridine; pyrimidine; indole;  
piperidine; morpholine; and thiomorpholine.

5

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20

As used in this application, the term "alkyl" represents an unsubstituted or substituted (by a halogen, nitro,  $\text{CONH}_2$ ,  $\text{COOH}$ ,  $\text{O-C}_{1-6}$  alkyl,  $\text{O-C}_{2-6}$  alkenyl,  $\text{O-C}_{2-6}$  alkynyl, hydroxyl, amino, or  $\text{COOQ}$ , wherein Q is  $\text{C}_{1-6}$  alkyl;  $\text{C}_{2-6}$  alkenyl;  $\text{C}_{2-6}$  alkynyl) straight chain, branched chain or cyclic hydrocarbon moiety (e.g. isopropyl, ethyl, fluoroethyl or cyclopropyl). The term alkyl is also meant to include alkyls in which one or more hydrogen atoms is replaced by an halogen, more preferably, the halogen is fluoro (e.g.  $\text{CF}_3$ - or  $\text{CF}_3\text{CH}_2$ -).

25

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35

The terms "alkenyl" and "alkynyl" represent an alkyl containing at least one unsaturated group (e.g. allyl).

The term "hydroxy protecting group" is well known in the field of organic chemistry. Such protecting groups may be found in T. Greene, Protective Groups In Organic Synthesis, (John Wiley & Sons, 1981). Example of hydroxy protecting groups include but are not limited to acetyl-2-thioethyl ester, pivaloyloxymethyl ester and isopropylloxycarbonyloxymethyl ester.

40

When there is a sulfur atom present, the sulfur atom can be at different oxydation level, S, SO, or  $\text{SO}_2$ . All such oxidation level are within the scope of the present invention.

45

It will be appreciated that the amount of pharmaceutical combination according to the invention required for use in treatment will vary not only with the particular compound

50

55

5 selected but also with the route of administration, the  
nature of the condition for which treatment is required  
and the age and condition of the patient and will be  
ultimately at the discretion of the attendant physician or  
10 5 veterinarian. In general however a suitable dose will be  
in the range of from about 0.1 to about 750 mg/kg of body  
weight per day, preferably in the range of 0.5 to 500  
mg/kg/day, most preferably in the range of 1 to 300  
15 mg/kg/day.

10  
The desired dose may conveniently be presented in a single  
20 dose or as divided dose administered at appropriate  
intervals, for example as two, three, four or more doses  
per day.

15  
The compounds of formula (1), (1a) and (1b) present in the  
pharmaceutical combination of the present invention are  
either additive or synergistic with the additional  
30 therapeutic agents in the combination and/or remove the  
20 cytotoxic effects of the other components.

35  
The pharmaceutical combination according to the present  
invention is conveniently administered in unit dosage  
form; for example containing 10 to 1500 mg, conveniently  
25 20 to 1000 mg, most conveniently 50 to 300 mg of active  
40 ingredient per unit dosage form.

45  
Ideally the active ingredient should be administered to  
achieve peak plasma concentrations of the active compound  
30 of from about 1 to about 75 $\mu$ M, preferably about 2 to 50  
 $\mu$ M, most preferably about 3 to about 30  $\mu$ M. This may be

5 achieved, for example, by the intravenous injection of a  
0.1 to 5% solution of the active ingredient, optionally in  
saline, or orally administered as a bolus containing about  
1 to about 500 mg of the active ingredient. Desirable  
10 blood levels may be maintained by a continuous infusion to  
provide about 0.01 to about 5.0 mg/kg/hour or by  
intermittent infusions containing about 0.4 to about 15  
15 mg/kg of the active ingredient.

10 The combinations referred to above may conveniently be  
presented for use in the form of a pharmaceutical  
20 formulation and thus pharmaceutical formulations  
comprising a combination as defined above together with a  
pharmaceutically acceptable carrier therefor comprise a  
25 further aspect of the invention.

The individual components of such combinations may be  
administered either sequentially or simultaneously in  
30 separate or combined pharmaceutical formulations.

20 When the compound (I) and (Ia) or a pharmaceutically  
acceptable salts thereof is used in combination with a  
35 second therapeutic agent active against the same virus the  
dose of each compound may be either the same as or differ  
25 from that when the compound is used alone. Appropriate  
40 doses will be readily appreciated by those skilled in the  
art.

The advantageous effects of the combination of the  
45 30 compounds of formula (1), (1a) and/or (1b) and the

5 additional therapeutic agents are realized over a wide ratio. For example 1:250 to 250:1,

10 In one embodiment, the ratio of the compounds of formula (1), (1a) and/or (1b) to the additional therapeutic agents in the present invention is between 1:50 to 50:1.

15 In one embodiment, the ratio of the compounds of formula (1), (1a) and/or (1b) to the additional therapeutic agents in our invention is between 1:20 to 20:1.

20 In a further embodiment, one may use from about 1:1 to about 1:15 of compounds of the invention:second therapeutic agent. In a further embodiment, one may use  
25 from about 1:1 to about 1:10 of compounds of the invention:second therapeutic agent. In a further embodiment, one may use from about 1:1 to about 1:5 of compounds of the invention:second therapeutic agent. In a  
30 further embodiment, one may use from about 1:1 to about 1:3 of compounds of the invention:second therapeutic agent. If a further therapeutic agent is added, ratios will be adjusted accordingly.

35 While it is possible that, for use in therapy, a compound of the invention may be administered as the raw chemical  
40 it is preferable to present the active ingredient as a pharmaceutical formulation. The invention thus further provides a pharmaceutical formulation comprising a compound of formula (1), (1a) and (1b) or a  
45 30 pharmaceutically acceptable derivative thereof together with one or more pharmaceutically acceptable carriers



5           therefor and, optionally, other therapeutic and/or  
prophylactic ingredients. The carrier(s) must be  
"acceptable" in the sense of being compatible with the  
10           other ingredients of the formulation and not deleterious  
5 to the recipient thereof.

15           Pharmaceutical formulations include those suitable for  
oral, rectal, nasal, topical (including buccal and sub-  
lingual), transdermal, vaginal or parenteral (including  
10 intramuscular, sub-cutaneous and intravenous)  
administration or in a form suitable for administration by  
20 inhalation or insufflation. The formulations may, where  
appropriate, be conveniently presented in discrete dosage  
units and may be prepared by any of the methods well known  
25 in the art of pharmacy. All methods include the step of  
bringing into association the active compound with liquid  
carriers or finely divided solid carriers or both and  
then, if necessary, shaping the product into the desired  
30 formulation.

20           Pharmaceutical formulation suitable for oral  
administration may conveniently be presented as discrete  
35 units such as capsules, cachets or tablets each containing  
a predetermined amount of the active ingredient; as a  
25 powder or granules; as a solution, a suspension or as an  
40 emulsion. The active ingredient may also be presented as a  
bolus, electuary or paste. Tablets and capsules for oral  
administration may contain conventional excipients such as  
binding agents, fillers, lubricants, disintegrants, or  
45 wetting agents. The tablets may be coated according to  
30 methods well known in the art. Oral liquid preparations

5 may be in the form of, for example, aqueous or oily  
suspensions, solutions, emulsions, syrups or elixirs, or  
may be presented as a dry product for constitution with  
10 water or other suitable vehicle before use. Such liquid  
5 preparations may contain conventional additives such as  
suspending agents, emulsifying agents, non-aqueous  
vehicles (which may include edible oils), or  
15 preservatives.

10 The pharmaceutical combination according to the invention  
may also be formulated for parenteral administration (e.g.  
20 by injection, for example bolus injection or continuous  
infusion) and may be presented in unit dose form in  
ampoules, pre-filled syringes, small volume infusion or in  
25 multi-dose containers with an added preservative. The  
compositions may take such forms as suspensions,  
solutions, or emulsions in oily or aqueous vehicles, and  
may contain formulatory agents such as suspending,  
30 stabilizing an/or dispersing agents. Alternatively, the  
20 active ingredient may be in powder form, obtained by  
aseptic isolation of sterile solid or by lyophilisation  
from solution, for constitution with a suitable vehicle,  
35 e.g. sterile, pyrogen-free water, before use.

25 For topical administration to the epidermis, the  
40 pharmaceutical combination according to the invention may  
be formulated as ointments, creams or lotions, or as a  
transdermal patch. Such transdermal patches may contain  
penetration enhancers such as linalool, carvacrol, thymol,  
45 citral, menthol and t-anethole. Ointments and creams may,  
30 for example, be formulated with an aqueous or oily base

5 with the addition of suitable thickening and/or gelling  
agents. Lotions may be formulated with an aqueous or oily  
base and will in general also contain one or more  
10 emulsifying agents, stabilizing agents, dispersing agents,  
5 suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the  
15 mouth include lozenges comprising active ingredients in a  
flavored base, usually sucrose and acacia or tragacanth;  
10 pastilles comprising the active ingredient in an inert  
base such as gelatin and glycerin or sucrose and acacia;  
20 and mouthwashes comprising the active ingredient in a  
suitable liquid carrier.

15 Pharmaceutical formulations suitable for rectal  
administration wherein the carrier is a solid are most  
preferably presented as unit dose suppositories. Suitable  
carriers include cocoa butter and other materials commonly  
30 used in the art, and the suppositories may be conveniently  
20 formed by admixture of the active compounds with the  
softened or melted carrier(s) followed by chilling and  
shaping in moulds.

Formulations suitable for vaginal administration may be  
25 presented as pessaries, tampons, creams, gels, pastes,  
40 foams or sprays containing in addition to the active  
ingredient such carriers as are known in the art to be  
appropriate.

45 30 For intra-nasal administration the pharmaceutical  
combination according to the invention may be used as a

5 liquid spray or dispersible powder or in the form of  
drops. Drops may be formulated with an aqueous or non-  
aqueous base also comprising one more dispersing agents,  
10 solubilising agents or suspending agents. Liquid sprays  
5 are conveniently delivered from pressurized packs.

For administration by inhalation the pharmaceutical  
15 combination according to the present invention are  
conveniently delivered from an insufflator, nebulizer or a  
10 pressurized pack or other convenient means of delivering  
an aerosol spray. Pressurized packs may comprise a  
20 suitable propellant such as dichlorodifluoromethane,  
trichlorofluoromethane, dichlorotetrafluoroethane, carbon  
dioxide or other suitable gas. In the case of a  
25 15 pressurized aerosol the dosage unit may be determined by  
providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or  
30 insufflation, the pharmaceutical combination according to  
20 the invention may take the form of a dry powder  
composition, for example a powder mix of the compound and  
a suitable powder base such as lactose or starch. The  
35 powder composition may be presented in unit dosage form  
in, for example, capsules or cartridges or e.g. gelatin or  
25 blister packs from which the powder may be administered  
40 with the aid of an inhalator or insufflator.

When desired the above described formulations adapted to  
45 give sustained release of the active ingredient may be  
30 employed.

5 The following examples are provided to illustrate various  
embodiments of the present invention and shall not be  
considered as limiting in scope.

#### 10 5 The Compounds

The compounds DXG, DAPD, DXG 5'-triphosphate (DXG-TP), (+)  
enantiomer of -D-1',3'-dioxolane guanosine, and  
15 lamivudine were synthesized at BioChem Pharma. as  
previously described (Belleau et al., 1989., Design and  
10 activity of a novel class of nucleoside analogs effective  
against HIV-1. Internatl. Conference on AIDS, Montreal  
20 (Quebec) Canada, June 4-9. ; Siddiqui et al., 1993,  
Bioorg. Med. Chem. Lett. 3:1543-1546). All of the  
dioxolanyl nucleosides were enantiomerically pure.

#### 25 Cells and Viruses

Human cord blood mononuclear cells (CBMCs) and peripheral  
30 blood mononuclear cells (PBMC) were obtained from HIV-1  
negative and hepatitis B virus negative donors (Department  
of Obstetrics, Jewish General Hospital, Montreal) and were  
isolated using Ficoll-Hypaque (Pharmacia) density gradient  
35 centrifugation. The CBMCs were then cultured under  
stimulation with 0.1 % (v / v) (5 mg / ml)  
25 Phytohemagglutinin (PHA ; Boehringer Mannheim, Montreal  
Canada) in RPMI-1640 medium (Gibco BRL Laboratories,  
40 Mississauga, Canada) containing 10% fetal calf serum (Flow  
Laboratories, Toronto, Canada), 2 mM glutamine, 100 U of  
penicillin, 100 mg of streptomycin and 15 U interleukin 2  
45 30 (IL-2, Boehringer Mannheim) per ml at 37°C and 5% CO<sub>2</sub> for 3-

5           4 days before used for antiviral assays (Rooke et al,  
1990, Virol. 176:205-215).

10           T-cell lines, i.e. MT-2, MT-4, H9 and Jurkat, and a  
5 monocyte cell line, i.e. U937, were obtained from either  
NIH AIDS Research and Reference Reagents (MD) or ATCC.  
These cells were used for antiviral and cytotoxicity  
15 studies and maintained as suspension cultures in RPMI-1640  
medium containing 10% fetal calf serum, 2 mM glutamine,  
20 100 U of penicillin, and 100 mg of streptomycin per ml.  
Other tumor cell lines, including Molt-4, HT-1080, DU145  
and HepG2 obtained from ATCC, and one normal cell line  
(human skin fibroblasts, HSF) obtained from Dr. M.  
Chrevette, (McGill University, Montreal, Canada), were  
25 also used for cytotoxicity assays and cultured in RPMI-  
1640 medium.

30           HIV-1<sub>IIIIB</sub> laboratory strain and HXB2-D recombinant of HIV-1  
were kindly supplied by R. C. Gallo (Institute of Human  
20 Virology, Baltimore, MD). Recombinant mutated HIV-1  
variants were prepared by site-directed mutagenesis as  
previously described (Gu et al., 1992. J. Virol. 66:12-19.  
35 and Gu, et al 1994, . Antimicrob. Agents Chemother. 38:275-  
281.). The recombinant viruses were generated by  
25 transfection of proviral DNA into MT-4 cells with  
Lipofectamine using the protocol recommended by the  
40 manufacturer (Gibco BRL, Montreal, Canada). HIV-1 clinical  
isolates were obtained by coculture of peripheral blood  
lymphocytes from HIV-1 infected individuals with the  
45 CBMCs, and then propagated on CBMCs in the absence of  
30 drugs as described by Salomon et al. (1994, J. Clin.

Microbiol. 32:2000-2002). Stock viruses were prepared from clarified culture supernatants by centrifugation and stored at -70°C. The viruses were titrated by limited dilution with a 4-fold serial dilution.

#### 5 Antiviral assays

Anti-HIV-1 activities of DXG and its prodrug DAPD were assessed by employing different HIV-1 variants and types of cells. Most experiments were performed with a laboratory strain HIV-1<sub>IIIB</sub>. A number of recombinant drug-resistance variants, and low passage clinical isolates from individuals who had received long-term anti-HIV therapy were also used to evaluate the effects of these two compounds. The methods used to assess the antiviral effect of the compounds have been previously described (Gu et al., 1992, J. Virol. 66:12-19. and Gu, et al 1994, Antimicrob. Agents Chemother. 38:275-281, . Rando et al., 1995; J. Biol. Chem. 270:1754-1760, Salomon et al, 1994, J. Clin. Microbiol. 32:2000-2002). The cells were incubated with virus using the indicated multiplicity of infection (MOI) for 2-3 hrs. The MOI used for each experiment was dependent upon the cell line and virus strain used, and was generally in the range of 0.005 to 0.5. For example, in assays performed using the established cell line MT-2, HIV-1<sub>IIIB</sub> at an MOI of 0.005 was used to infect cells. The unbound virus was then removed by washing the cells, followed by plating the cells into a 96-well plate. The infected cells were cultured in the presence of a serial concentrations of the test compound for 5-7 days. The anti-HIV-1 efficacy was determined by testing for HIV-1 RT activity in the cell culture supernatants. All assays were performed in duplicate and

at least two independent experiments were performed. Anti-HIV-1 efficacy of DXG and DAPD was compared to the approved anti-HIV-1 drugs AZT and / or lamivudine controls in each individual experiment. The susceptibilities of the HIV-1 variants to antiretroviral agents are expressed as the mean of the EC<sub>50</sub> determinations.

Combination effects between DXG and approved anti-HIV-1 agents were assessed in CBMCs using HIV-1<sub>IIIB</sub>. The combinations of the inhibitors was performed with a checker board cross. The antiviral effects were monitored through testing RT activity in the culture supernatants at day 7. The data was analyzed according to the method described by Chou and Talalay (Chou and Talalay, 1984, Adv. Enzyme Regul. 22:27-55). The combination indexes (CIs) of DXG with other anti-HIV-1 agents were calculated by using CalcuSyn program (Biosoft, Cambridge, UK). Theoretically, CI of 1 indicates additive effect; CIs of >1 and <1 stand for antagonism and synergism between the drugs combined, respectively.

#### Cytotoxicity Analysis

The cellular toxicity of the BCH compounds were assessed on various cells using [<sup>3</sup>H]-thymidine uptake. The various cells, including Molt-4, HT1080, DU-145, HepG 2 and HSF, were plated at a concentration of 1-2 x 10<sup>3</sup> cells per well (96 well plates). However, PHA-stimulated PBMCs were cultured at a concentration of 4 x 10<sup>4</sup>. After a 24 hr incubation period, 10-fold serial diluted compounds (10<sup>-4</sup> M to 10<sup>-10</sup> M) were added to the culture medium and the cells were further incubated for 72 hrs. [<sup>3</sup>H]-thymidine was added



5 during the final 18 hr incubation period. After incubation  
with the [<sup>3</sup>H]-thymidine, the cells were washed once with  
PBS, treated with trypsin if the cells were adherent, and  
then resuspended in water (hypotonic lysing of cells). The  
10 5 cellular extract was applied directly to a Tomtec  
Harvester 96. Using this instrument the extracted DNA is  
adsorbed onto filters, washed and the incorporated [<sup>3</sup>H]-  
15 thymidine is then counted. The 50% cytotoxic concentration  
(CC<sub>50</sub>) was determined by comparing the radioactive counts  
10 per minute of the samples in the presence of the compounds  
against the control.

20 The cellular toxicity of the compounds was also tested by  
WST-1 staining through assessing proliferation of MT-2,  
25 H9, Jurkat, U937, and CBMCs. The established cell lines  
were cultured in RPMI medium in 96-well plates at a  
density of 5 x 10<sup>4</sup> cells / well while CBMCs were plated at  
a concentration of 0.5 x 10<sup>6</sup> / well. A 10-fold serial  
30 diluted (10<sup>-4</sup>-10<sup>-7</sup> M) compound was added at day zero. At day  
20 4, the cells were passaged by changing half medium  
containing appropriately diluted compound. The cell  
activities were assessed at day 7 using the WST-1 reagent  
35 (Boehringer Mannheim) following the protocol provided by  
the supplier.

25

#### Reverse Transcriptase enzyme assays

40 Wild type (wt) version of recombinant HIV-1 RT were  
expressed with a histidine tag in an E. coli protein  
expression system. The enzymes was purified up to 95%  
45 30 homogeneity as described by Gu et al. (1994, J. Biol.

Chem. 269:28118-28122., and 1995, Proc. Natl. Acad. Sci. USA 92:2760-2764.).

RT inhibition assay. The inhibition of HIV-1 RT RNA dependent DNA polymerase activity of DXG-TP was assessed under steady-state enzymatic kinetics by employing homopolymeric RNA templates / DNA primers (T / P) and a heteropolymeric RNA template / DNA primer. The heteropolymeric RNA template contains the HIV-1 primer binding sequence, U5 and R regions (designated as HIV-PBS). The HIV-PBS RNA was in vitro transcribed from a plasmid DNA as described previously (Gu et al., 1994, J. Biol. Chem. 269:28118-28122.). The oligodeoxynucleotide primer (dPR) is an 18-mer (5'-GTCCCTGTTCTGGGCGCCA-3') which is complementary to the HIV-1 primer binding sequence. The complex of HIV-PBS and dPR T / P was prepared by mixing a 1:2 ratio in 50 mM Tris-HCl (pH 7.8) containing 60 mM KCl, heating to 95°C for 2 min, and then slowly cooling down to room temperature (Gu et al., 1994, J. Biol. Chem. 269:28118-28122). The reverse transcription reaction contained final concentrations of 50 mM Tris-HCl, pH 7.8, 60 mM KCl, 10 mM MgCl<sub>2</sub>, 0.1 U / ml homopolymeric T / P and 5 µM [<sup>3</sup>H]dNTP substrate or 25 nM HIV-PBS / dPR and 5 µM each of dTTP, dCTP, dGTP and [ -<sup>32</sup>P]dATP in 100 µl. Reactions were incubated for 30 min at 37°C in the presence or absence of dideoxynucleoside triphosphate inhibitors as described by Gu et al., (1994, J. Biol. Chem. 269:28118-28122.).

Inhibition of dNTP incorporation / chain termination. The effect of DXG-TP on RT activity was also assessed using

5 the chain termination / dNTP incorporation assay in which  
inhibition of nascent DNA synthesis (chain termination)  
was monitored based upon cDNA synthesis as previously  
described (Arts et al., 1993; J. Biol. Hem. 269:14672-  
10 14680 and Gu et al., 1995, Proc. Natl. Acad. Sci. USA  
92:2760-2764). HIV-PBS RNA template and dPR DNA primer  
were used in this system. The RT reactions were performed  
15 in 20 µl volumes containing 50 mM Tris (pH 7.8), 75 mM  
KCl, 10 mM MgCl<sub>2</sub>, 100 µM of dNTPs. The HIV-PBS RNA template  
10 (50 nM) and [ <sup>32</sup>P ]-ATP labeled oligodeoxynucleotide primer  
(125 nM) were included in the reaction. The mixture was  
20 first denatured at 85°C for 2 minutes, then cooled to 55°C  
for 8 min, and finally cooled to 37°C at which time  
recombinant HIV RT was added (42.5 nM). The reactions were  
25 allowed to proceed at 37°C for 60 min in the presence or  
absence of inhibitors. The transcribed DNA products were  
separated on a 5% denaturing polyacrylamide gel and  
visualized by exposure to X-ray film.

#### 20 Determination of HIV-1 RT genotype

To determine the RT genotype of the HIV-1 clinical  
isolates, proviral DNA of each isolate was extracted from  
35 infected CD4<sup>+</sup> T-cells or CBMCs as previously reported (Gu  
et al., 1992). The complete RT coding regions were  
25 amplified by PCR employing a primer pair consisting of the  
up-stream primer RT01 (5'-GTAGAATTCTGTTGACTCAGATTGG-3'),  
40 and the down-stream primer RT02 (5'-  
GATAAGCTTGGCCTTATCTATTCCAT-3') as previously described  
(Gu et al., 1992, J. Virol. 66:12-19.). The amplified  
45 30 fragment were 1.7 kb and contained the complete RT coding  
sequence. The PCR amplified fragments were separated by

5 agarose gel electrophoresis and purified by Qiaquick Gel  
Extraction kit (Qiagen, Mississauga, Ontario, Canada). The  
purified PCR product was directly sequenced using primer  
10 RTS (5'-CCAAAAGTTAAACAATGGC-3') which is located at the 5'  
5 portion of the RT coding region (nucleotide 2603-2621 of  
HXB2-D co-ordinates). The nucleic acid sequence of RT was  
sequenced and compared with the published sequences of  
15 wild-type HIV-1 strains.

#### 10 Example 1

##### 20 Anti-HIV-1 efficacy of dioxolanyl analogues in various cells

Since anabolic efficiency of nucleoside analogues, i.e.  
phosphorylation and prodrug conversion, is mediated by the  
25 related cellular enzymes which activities depend on type  
of cells, we assessed the anti-HIV-1 efficacy of the  
dioxolanyl compounds, DXG and DAPD, in human CBMCs and a  
variety of human T and monocyte cell lines. All data in  
30 these assays were obtained using HIV-1<sub>IIIB</sub>. Approved anti-  
20 HIV agents, AZT and lamivudine, were used in each of the  
experiments as controls. Table 1 summarizes the data of  
the antiviral efficacy of the compounds while Figure 1  
35 shows a dose response curve for the inhibition of HIV-1 in  
MT-2 cells. Generally, The dioxolanyl compounds had the  
25 same efficacy in CBMCs and in T-cell lines. For example,  
EC<sub>50</sub>s were 0.046  $\mu$ M and 0.085  $\mu$ M for DXG tested in CBMCs  
and in MT-2 cells, respectively. EC<sub>50</sub>s for DAPD were  
usually 5-20-fold higher than those for DXG in various  
40 cells, e.g. 0.97  $\mu$ M and 0.54  $\mu$ M EC<sub>50</sub>s for this prodrug in  
45 CBMCs and in MT-2 cells, respectively. In addition,  
30 comparing with the anti-HIV-1 efficacy of the approved

5 agents, DXG were generally equivalent to the efficacy of  
lamivudine in the various cells, but approximately 5-10-  
fold less than that of AZT (Table 1).

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TABLE 1.  
Inhibitory effects of DXG and DAPD on HIV-1 replication<sup>a</sup>

Cell	EC <sub>50</sub> , $\mu$ M of mean values $\pm$ SD (No. of experiments)			
	DXG	DAPD	lamivudine	AZT
CBMCs	0.046 $\pm$ 0.017 (3)	0.97 $\pm$ 0.092 (2)	0.023 $\pm$ 0.011 (4)	0.0051 $\pm$ 0.003 (4)
MT-2	0.085 $\pm$ 0.026 (7)	0.54 $\pm$ 0.29 (6)	0.091 $\pm$ 0.08 (6)	0.0076 $\pm$ 0.0044 (5)
MT-4	0.051 $\pm$ 0.009 (2)	0.94 $\pm$ 0.028 (2)	0.056 $\pm$ 0.014 (2)	0.008 $\pm$ 0.005 (2)
Jurkat	0.34 $\pm$ 0.14 (2)	1.37 $\pm$ 0.72 (2)	0.53 $\pm$ 0.02 (2)	0.011 $\pm$ 0.007 (2)
H9	0.06 $\pm$ 0.05 (2)	0.075 $\pm$ 0.04 (2)	ND <sup>b</sup>	0.041 $\pm$ 0.05 (2)
U937 <sup>c</sup>	0.41	1.3	ND	0.025

<sup>a</sup> All assays were performed using laboratory strain HIV-1<sub>lab</sub>.

<sup>b</sup> ND, not determined

<sup>c</sup> Single experiment in duplicate.

5 We also compared the antiviral efficacy between (-) and (+) enantiomers of -D-1',3'-dioxolane guanosine. Our results showed that the (+) enantiomer, with 0.7  $\mu$ M of EC<sub>50</sub>, had less antiretroviral activity than its (-) enantiomer partner tested in MT-2 cells.

#### 10 Example 2

#### 15 Susceptibility of recombinant drug-resistance HIV-1 variants to DXG and DAPD

Recombinant HIV-1 variants carrying drug-resistant mutation(s) were employed to test the cross-resistance phenotype of DXG and DAPD in CBMCs and MT-2 cells. Table 2 summarizes the background of the variants and their sensitivities to the dioxolanylpurine compounds as well as the approved NRTIs in CBMCs. These mutants consist of those seen for the commonest RT inhibitor-resistance HIV-1 variants generated either in vitro selection or from patients undergoing anti-retroviral therapy with NRTIs, such as AZT, lamivudine, 2',3'-dideoxyinosine (ddI) and ddC. All of the recombinants are derived from HXB2-D. The data in Table 2 indicated that the variants of HIV-1 carrying ddI-, ddC- or lamivudine-resistance mutations, i.e. 65K, 74V, and 184V substitutions in the RT gene, had minimal (2 to 5-fold) decreased sensitivity to DXG and DAPD referred to the wt HXB2-D. In addition, the variant bearing mutations of 41L and 215Y combined with 184V, which has a high-level resistance to lamivudine but reversed sensitivity to AZT, had approximate 2-fold decreased sensitivity to DXG which was similar to the 184V single mutated recombinant.

45 In contrast, AZT resistant virus, i.e. the recombinant carrying 41L, 70R, 215Y and 219Q multiple substitutions in

5 the RT, remained completely sensitive to DXG and DAPD both  
in CBMCs (Table 2), this was also observed in MT-2 cells.  
In addition, antiviral assays also demonstrated that these  
10 dioxolanyl nucleoside analogues were sensitive against  
NNRTI-resistant and protease inhibitor-resistant variants  
(Table 2).



**TABLE 2.**  
**Effect of DXG and DAPD on recombinant drug-resistant HIV-1**

Recombinant	Resistance to:	EC <sub>50</sub> (μM) in CBMCs of:			
		DXG	DAPD	lamivudine	AZT
HXB2-D*					
Wt	wt	0.21±0.05	1.1±0.16	0.041±0.012	0.0041±0.001
65R	ddl, ddC, lamivudine PMEA	1.2±0.2	6.5±2.1	0.36±0.17	0.003±0.0003
74V	ddl	1.3±0.8	6.6±3.4	0.12±0.07	0.006±0.0002
184V	lamivudine, ddl, ddC	0.44±0.07	2.1±0.20	>50 <sup>b</sup>	0.0027±0.000
41L/70R/215Y/219Q	AZT	0.24±0.02	1.25±0.59	0.062±0.020	0.082±0.08
41L/215Y/184V	lamivudine	0.41±0.10	2.3±0.05	>50 <sup>b</sup>	0.006±0.004
106A/181C <sup>c</sup>	NNRTIs	0.05±0.007	ND <sup>d</sup>	ND	0.03±0.005
10R/46I/63P/82T/84V <sup>e</sup>	sacquinavir	0.12±0.03	1.37±0.06	ND	0.0032±0.001

\* The recombinant viruses are wild-type (wt) and mutants harboring the substitution(s) in the RT indicated.

<sup>b</sup> The highest concentration of lamivudine used in these assays was 50 μM.

<sup>c</sup> EC<sub>50</sub> for nevirapine was > 10 μM.

<sup>d</sup> ND, not determined

<sup>e</sup> Protease genotype; EC<sub>50</sub> for saquinavir was 0.075±0.011 μM.

## Example 3.

## Susceptibility of HIV-1 clinical isolates to DXG and DAPD

The population of HIV-1 in infected individual is 5 quasispecies and the sensitivity of these different viruses found in clinical isolates to antiviral chemotherapy might be quite variable. In addition, HIV-1 isolates obtained from patients receiving long-term antiretroviral therapy might behave differently from 10 cloned virus containing genetically engineered mutations in the RT gene. For these reasons, clinical isolates of HIV-1 from antiviral naive and drug-treated patients were assayed in PHA-stimulated CBMCs for their sensitivity to DXG and DAPD accompanied with approved 15 antiretroviral agents. The genotype of the HIV-1 clinical isolates were determined as described above.

Table 3 shows the summary of the recent therapy history for patients from which the HIV-1 isolates were 20 obtained, the RT genotype of the isolates, and their sensitivity to the indicated anti-HIV agents. Four isolates, i.e. number 3887, 4246, 4877 and 4526, were 35 sensitive to AZT and/or lamivudine, or marginal decreased sensitivity to one of these two drugs, 25 referred to  $EC_{50}$ s obtained with recombinant variants (Table 2 ). These isolates were obtained from HIV-1 infected individuals who were either anti-HIV therapy naive or treated with the RT inhibitors. The isolates 3887 carried 184V substitution mixed with wt 184M, and 45 the isolate 4877 had 41L mutation in the RTs. As shown 30 in the Table 3, the  $EC_{50}$ s obtained using these four

isolates for both DXG and prodrug DAPD are comparable to those observed with the wt strains, i.e. HIV-1<sub>III<sub>B</sub></sub> and HXB2-D assessed in CBMCs (see Tables 1 and 2).

5 The isolates 3350 and 4205, from patients who had received lamivudine therapy, carried 184V mutation in their RTs and were high-degree resistance to lamivudine but remained sensitive to AZT. Consistent with the results obtained using the recombinant variants 10 (Table 2), these 184V mutated isolates had an approximate 5-fold decreased susceptibility to DXG and DAPD when compared to the lamivudine and AZT sensitive isolates (Table 3).

TABLE 3.  
Susceptibility of HIV-1 Isolates from patients treated with nucleoside analogs to DXG and DAPD

Viral Isolate	Antiviral Therapy (week)	RT genotype	EC <sub>50</sub> (μM) in CBMCs of.		
			DXG	DAPD	AZT
3887	Lamivudine (24)	184MV	0.18±0.007	0.19±0.12	0.11±0.1
4246	AZT (104)	wt <sup>a</sup>	0.12±0.08	0.41±0.21	0.023±0.02
4526 <sup>c</sup>	Naive	ND <sup>b</sup>	0.055±0.027	0.85±0.071	ND
4877 <sup>d</sup>	Naive	41L	0.045±	0.26±	0.015±
3350	Lamivudine (12)	184V	0.65±0.33	3.3±1.2	>100
4205	Lamivudine (52)	184V	1.1±1.19	4.1±0.35	>100
4242	AZT	41L/70R/215Y	0.21±0.007	0.88±0.3	0.009
4833 <sup>c</sup>	Saquinavir (48)	ND	0.17±0.06	0.63±0.38	ND
4924 <sup>d</sup>	AZT/nevirapine (26)	41L/103N	0.02±	0.17±	ND

<sup>a</sup> wt, wild-type.

<sup>b</sup> ND, not determined.

<sup>c</sup> Both RT and protease genotypes were not determined. EC<sub>50</sub>s for saquinavir were 0.0063±0.0039 μM against isolate 4526 and 0.11±0.028 μM against isolate 4833.

<sup>d</sup> EC<sub>50</sub>s for nevirapine were 0.065±0.001 μM against isolate 4877 and >10 μM against isolate 4924.

5           However, the isolate 4242 which was obtained from  
patient treated with AZT and carried AZT-resistance  
10           mutations, i.e. 41L / 70R / 215Y in RT, had decreased  
sensitivity to AZT as expected, but remained sensitive  
5           to DXG, DAPD as well as lamivudine. Assay used an  
NNRTI-resistant strain 4924 isolated from an individual  
received AZT and nevirapine combination therapy, which  
15           carried 41L / 103N mutations in the RT and had >10  $\mu$ M  
EC<sub>50</sub> for nevirapine, was sensitive to the dioxolane  
10           nucleoside analogues (Table 3). In addition, the  
dioxolane compounds was also observed to be completely  
20           sensitive to the protease inhibitor-resistance isolate  
4833 which was obtained from an individual received 48-  
week saquinavir therapy and had about 20-fold decreased  
25           sensitivity to this protease inhibitor compared to the  
baseline isolate 4526 (Table 3).

#### 30           Example 4 .

#### 20           Combination effects of DXG with approved anti-HIV-1 agents

35           DXG, the active form of its prodrugs, was assessed  
through combinations with the approved anti-HIV-1  
25           agents, i.e. NRTIs (AZT and lamivudine) and NNRTI  
(nevirapine) to inhibit HIV-1 replication in CBMCs  
40           against the laboratory strain HIV-1<sub>ITB</sub>. The combination  
indexes (Cis) of DXG combined with approved anti-HIV-1  
agents are summarized in Table 4. The CIs were  
45           calculated at several effective concentration levels,  
30           i.e. EC<sub>50</sub>, EC<sub>75</sub>, EC<sub>90</sub> and EC<sub>95</sub>, in different molar ratios

5 of the combined drugs. The most of the CIs were between  
0.4-0.8 in the case of DXG combined with either  
lamivudine or nevirapine, which suggest that DXG had  
moderate synergism with these two anti-HIV-1 agents.  
10 However, this compounds had greater synergism with  
thymidine analogue AZT with CIs between 0.3-0.8 at  $EC_{50}$   
and less than 0.3 at higher EC levels which indicates a  
strong synergism.  
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TABLE 4.  
Combination effects of DXG with approved anti-retroviral agents

Drug Combination	Molar ratio	CI at inhibition level in CBMCs			
		EC <sub>50</sub>	EC <sub>75</sub>	EC <sub>90</sub>	EC <sub>95</sub>
DXG / AZT	10 : 1	0.61	0.27	0.12	0.07
	20 : 1	0.57	0.29	0.15	0.10
	40 : 1	0.67	0.30	0.14	0.08
	80 : 1	0.78	0.37	0.18	0.11
DXG / lamivudine	1 : 1.6	0.79	0.59	0.47	0.41
	1.25 : 1	0.82	0.58	0.46	0.40
	2.5 : 1	0.74	0.52	0.42	0.38
	5 : 1	0.65	0.69	0.87	1.08
DXG / nevirapine	1.25 : 1	0.88	0.65	0.52	0.46
	2.5 : 1	0.90	0.64	0.52	0.47
	5 : 1	0.87	0.54	0.38	0.32
	10 : 1	0.95	0.57	0.38	0.31

## Example 5 .

## Cellular toxicity

DXG and DAPD along with lamivudine and AZT were tested for their effect on cell proliferation using both [<sup>3</sup>H]-thymidine uptake and cell proliferation (WST-1) assays. Human PBMC and a number of established solid and leukemic cancer cell lines (Molt-4, HT-1080, DU-145, HepG2) and one normal cell line (human skin fibroblasts, HSF) were used in the [<sup>3</sup>H]-thymidine uptake study. The results from these studies showed that DXG and DAPD were non toxic to the cell proliferation up to a concentration of 500  $\mu$ M in the [<sup>3</sup>H]-thymidine incorporation experiment (Table 5). In the same experiments, CC<sub>50</sub>s for AZT and ddC had less than 10  $\mu$ M in the cells tested. In addition, DXG did not have toxicity to human CBMCs and several cell lines, i.e. MT-2, H9, Jurkat and U937, up to 100  $\mu$ M, the highest concentrations tested in the WST-1 cell viability assay compared to the 74  $\mu$ M and 29  $\mu$ M of CC<sub>50</sub> for both AZT and ddC, respectively . Thus, DXG and DAPD were less cytotoxic than AZT and ddC in these assay systems.



TABLE 5.  
Effect of nucleoside analogs on cell proliferation in [<sup>3</sup>H]-thymidine uptake assay

Cell	CC <sub>50</sub> (μM)				ddC
	DXG <sup>a</sup>	DAPD <sup>a</sup>	lamivudine	AZT	
PBMC	>500	>500	ND <sup>b</sup>	ND	32.5
Molt-4	>500	>500	ND	3	2
HT-1080	>500	>500	>500	5	2
HepG2	>500	≥500	350	3	7
DU145	>500	>500	>500	>10	5
HSF	≥500	>500 (350)	400	>10	ND

<sup>a</sup> The highest concentrations of DXG and DAPD used in these studies were 500 μM.

<sup>b</sup> ND, not determined.

## Example 6 .

## Inhibition of HIV-1 RT polymerase activity by DXG triphosphate

The DXG-TP would most likely be the antiviral active form for the diaminopurine dioxolane DAPD in vivo.. The inhibitory effect of DXG-TP on HIV-1 RT activity was assessed using various homopolymeric template / primers (T / P) and a heteropolymeric T / P, i.e. HIV-PBS / dPR. The results from these experiments demonstrated that the DXG-TP was a potent HIV-1 RT inhibitor with 0.012  $\mu\text{M}$   $\text{IC}_{50}$ , obtained using wt HIV-1 RT when complementary poly(rC).oligo(dG) T / P and dGTP substrate were used in the enzymatic reactions (Table 6). This value has approximately the same inhibitory efficiency as the parental dideoxyguanosine triphosphate (ddGTP). Similarly, DXG-TP and ddGTP were observed to have the same inhibition of RT when the poly(rC).oligo(dG) was replaced by heteropolymeric template / primer HIV-PBS / dPR (Table 6). In addition, the RT inhibition of DXG-TP was observed to be competition with natural substrate, i.e. the higher the concentration of dGTP, the lower the inhibitory effect of DXG-TP. However, as expected DXG-TP did not show any inhibition of HIV-1 RT activity up to 10  $\mu\text{M}$  when the non-complementary T / P poly(rA).oligo(dT) was used along with dTTP as the substrate. (Table 6).

TABLE 6.  
Inhibition of HIV-1 reverse transcriptase by DXG-TP and other dideoxynucleotide triphosphates

Template / primer	IC <sub>50</sub> (μM)		
	Substrate	DXG-TP	ddTTP
poly(rC).oligo(dG) <sub>12-18</sub>	dGTP	0.012±0.002	0.011±0.0007
poly(rA).oligo(dT) <sub>12-18</sub>	dTTP	>10 <sup>b</sup>	ND <sup>a</sup>
HIV-PBS / dPR	dNTPs <sup>c</sup>	0.062±0.007	0.074±0.008

<sup>a</sup> ND, not determined.  
<sup>b</sup> The highest concentration of inhibitor used in the inhibition study was 10 μM.  
<sup>c</sup> Each of the dATP, dCTP, dGTP and dTTP was 5 μM.

5 The chain elongation / termination assay provides a  
method to directly visualize the products of  
incorporation of dideoxynucleotide monophosphates into  
nascent DNA by monitoring the reaction products using  
10 5 polyacrylamide gel electrophoresis. The experiment was  
performed using wt HIV-1 RT, and HIV-PBS  
heteropolymeric template, and [<sup>32</sup>P]ATP labeled dPR  
primer in the presence of various concentrations of RT  
15 inhibitor. The concentrations of the inhibitors used  
10 were 0, 0.7, 2.2, 6.6, 20, and 60  $\mu$ M for DXG-TP, ddGTP  
and AZT-TP; 0, 1, 3, 10, 33, and 100  $\mu$ M for 3TC-TP; 0,  
20 0.005, 0.02, 0.08, 0.32 and 1.5  $\mu$ M for NNRTI  
nevirapine. Fig. 2 shows results of a chain elongation  
/ termination assay in which DXG-TP employed as HIV-1  
25 RT inhibitor compared with other NRTI triphosphates,  
i.e. ddGTP, AZT-TP and lamivudine-TP, and a NNRTI  
nevirapine. The bands at the top of the gel were full-  
length DNA products of the RT reaction. In the lanes  
30 which reactions were absence of RT inhibitors, the  
20 remaining bands which were shorter than the full-length  
products are pausing products due to the fact that HIV-  
1 RT is a processive enzyme. The extra bands (indicated  
35 by arrows as examples) which are merely observed in the  
lanes in the presence of dideoxynucleotide triphosphate  
25 inhibitors are chain termination products. DXG-TP  
together with other nucleotides tested, i.e. ddGTP,  
40 AZT-TP and lamivudine-TP, caused increasing chain  
termination but decreasing full-length products with  
raising inhibitor concentration. As comparison, NNRTI  
45 30 nevirapine was also used in the assay. In this case,  
nevirapine also showed the decrease of the full-length

5 DNA products, but there were no extra chain termination  
bands generated, as expected. These results reflect the  
different mechanisms of inhibition of the RT between  
10 NRTIs and NNRTIs.

5  
Furthermore, the pattern of chain-termination bands  
generated by incorporation of DXG-MP into elongating  
15 DNA strands were exactly the same as pattern of its  
parental dideoxyguanine (ddGMP) but different from  
10 thymidine and cytidine analogues, i.e. AZT-MP and  
lamivudine-MP incorporation (see Figure 2). Generally,  
20 the inhibitory effect of DXG-TP on RT activity in this  
cell-free assay, determined by the intensity of the  
chain-termination and full-length bands generated , was  
25 the same as ddGTP and AZT-TP at the same  
concentrations, but higher than lamivudine-TP.

## Claims

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What is claimed is

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1. A pharmaceutical combination useful for the treatment of viral infections comprising at least one of (-)- $\beta$ -D-2,6-diaminopurine-1,3- dioxolane ( $\beta$ -D-DAPD) and (-)- $\beta$ -D -1,3-dioxolane guanine ( $\beta$ -D-DXG) and at least one further therapeutic agent chosen from zidovudine, didanosine, zalcitabine, stavudine, lamivudine, nevirapine, delavirdine, efavirenz, indinavir, nelfinavir, saquinavir or ritonavir.

2. A pharmaceutical combination according to claim 1 wherein the  $\beta$ -D-dioxolane is at least 97% free of the corresponding (+) enantiomer.

3. A pharmaceutical combination according to claim 1 wherein at least one further therapeutic agent is chosen from zidovudine, lamivudine, nevirapine and combinations thereof.

4. A pharmaceutical combination according to claim 2 wherein at least one further therapeutic agent is chosen from zidovudine, lamivudine, nevirapine and combinations thereof.

5. A pharmaceutical combination according to anyone of claims 1 to 4 for use in medical therapy.

6. The pharmaceutical combination according to anyone of claims 1 to 4 for use in the treatment of HIV infection.

7. A pharmaceutical formulation comprising a pharmaceutical combination according to anyone of

5                   claims 1 to 4 with at least one pharmaceutically  
                  acceptable carrier or excipient.

10           8. A    pharmaceutical    formulation    comprising    a  
                  pharmaceutical combination according to claim 5 with  
                  at least one pharmaceutically acceptable carrier or  
                  excipient.

15           9. A    pharmaceutical    formulation    comprising    a  
                  pharmaceutical combination according to claim 6 with  
                  at least one pharmaceutically acceptable carrier or  
20           excipient.

              10. A pharmaceutical combination according to anyone  
                  of claims 1 to 4 wherein the antiviral active  
25           compounds and the therapeutic agents are present in a  
                  synergistic ratio.

              11. A pharmaceutical combination according to claim 5  
                  wherein the antiviral active compounds and the  
30           therapeutic agents are present in a synergistic  
                  ratio.

              12. A pharmaceutical combination according to claim 6  
35           wherein the antiviral active compounds and the  
                  therapeutic agents are present in a synergistic  
                  ratio.

40           13. A pharmaceutical combination according to claim 8  
                  wherein the antiviral active compounds and the  
                  therapeutic agents are present in a synergistic  
45           ratio.



5           14. A pharmaceutical combination according to claim 9  
          wherein the antiviral active compounds and the  
          therapeutic agents are present in a synergistic  
10           ratio.

          15. A pharmaceutical combination according to anyone  
          of claims 1 to 4 wherein the antiviral active  
15           compounds and the therapeutic agents are present in a  
          ratio between about 1:250 to about 250:1.

          16. A pharmaceutical combination according to anyone  
          of claims 1 to 4 wherein the antiviral active  
20           compounds and the therapeutic agents are present in a  
          ratio between about 1:50 to about 50:1.

          17. A pharmaceutical combination according to anyone  
25           of claims 1 to 4 wherein the antiviral active  
          compounds and the therapeutic agents are present in a  
          ratio between about 1:20 to about 20:1.

          18. A pharmaceutical combination according to claim 5  
          wherein the antiviral active compounds and the  
30           therapeutic agents are present in a ratio between  
          about 1:250 to about 250:1.  
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          19. A pharmaceutical combination according to claim 5  
          wherein the antiviral active compounds and the  
40           therapeutic agents are present in a ratio between  
          about 1:50 to about 50:1.

          20. A pharmaceutical combination according to claim 5  
45           wherein the antiviral active compounds and the  
          therapeutic agents are present in a ratio between  
          about 1:20 to about 20:1.

5           21. A pharmaceutical combination according to claim 6  
          wherein the antiviral active compounds and the  
          therapeutic agents are present in a ratio between  
10           about 1:250 to about 250:1.

          22. A pharmaceutical combination according to claim 6  
          wherein the antiviral active compounds and the  
15           therapeutic agents are present in a ratio between  
          about 1:50 to about 50:1.

          23. A pharmaceutical combination according to claim 6  
          wherein the antiviral active compounds and the  
20           therapeutic agents are present in a ratio between  
          about 1:20 to about 20:1.

          24. A pharmaceutical combination according to claim 8  
25           wherein the antiviral active compounds and the  
          therapeutic agents are present in a ratio between  
          about 1:250 to about 250:1.

30           25. A pharmaceutical combination according to claim 8  
          wherein the antiviral active compounds and the  
          therapeutic agents are present in a ratio between  
35           about 1:50 to about 50:1.

          26. A pharmaceutical combination according to claim 8  
          wherein the antiviral active compounds and the  
40           therapeutic agents are present in a ratio between  
          about 1:20 to about 20:1.

          27. A pharmaceutical combination according to claim 9  
45           wherein the antiviral active compounds and the  
          therapeutic agents are present in a ratio between  
          about 1:250 to about 250:1.

5           28. A pharmaceutical combination according to claim 9  
          wherein the antiviral active compounds and the  
          therapeutic agents are present in a ratio between  
10           about 1:50 to about 50:1.

          29. A pharmaceutical combination according to claim 9  
          wherein the antiviral active compounds and the  
15           therapeutic agents are present in a ratio between  
          about 1:20 to about 20:1.

          30. A method for the treatment of viral infections  
          comprising administering a therapeutically effective  
20           amount of at least one of (-)- $\beta$ -D-2,6-diaminopurine-  
          1,3- dioxolane( $\beta$ -D-DAPD) and (-)- $\beta$ -D -1,3-dioxolane  
          guanine( $\beta$ -D-DXG) and at least one further therapeutic  
25           agent chosen from zidovudine, didanosine,  
          zalcitabine, stavudine, lamivudine, nevirapine,  
          delavirdine, efavirenz, indinavir, nelfinavir,  
30           saquinavir or ritonavir to a subject in need of such  
          treatment.

          31. The method of claim 30 wherein the  $\beta$ -D-dioxolane is  
35           at least 97% free of the corresponding (+)  
          enantiomer.

          32. The method of claim 30 wherein at least one  
40           further therapeutic agent is chosen from zidovudine,  
          lamivudine, nevirapine and combinations thereof.

          33. The method of claim 31 wherein at least one  
45           further therapeutic agent is chosen from zidovudine,  
          lamivudine, nevirapine and combinations thereof.

- 5                   34. The method according to anyone of claims 30 to 33  
                  wherein the viral infection is an HIV infection.
- 10                   35. The method according to anyone of claims 30 to 33  
                  wherein the compounds and the other therapeutic  
                  agents are administered sequentially.
- 15                   36. The method according claim 34 wherein the  
                  compounds and the other therapeutic agents are  
                  administered sequentially.
- 20                   37. The method according to anyone of claims 30 to 33  
                  wherein the compounds and the other therapeutic  
                  agents are administered simultaneously.
- 25                   38. The method according to claim 34 wherein the  
                  compounds and the other therapeutic agents are  
                  administered simultaneously.
- 30                   39. The method according to anyone of claims 30 to 33  
                  wherein the antiviral active compounds and the  
                  therapeutic agents are present in a synergistic  
                  ratio.
- 35                   40. The method of claim 34 wherein the antiviral  
                  active compounds and the therapeutic agents are  
                  present in a synergistic ratio.
- 40                   41. The method of claim 35 wherein the antiviral  
                  active compounds and the therapeutic agents are  
                  present in a synergistic ratio.
- 45                   42. The method of claim 36 wherein the antiviral  
                  active compounds and the therapeutic agents are  
                  present in a synergistic ratio.

5           43. The method of claim 37 wherein the antiviral  
active compounds and the therapeutic agents are  
present in a synergistic ratio.

10           44. The method of claim 38 wherein the antiviral  
active compounds and the therapeutic agents are  
present in a synergistic ratio.

15           45. The method according to anyone of claims 30 to 33  
wherein antiviral active compounds and the  
therapeutic agents are present in a ratio between  
20           about 1:250 to about 250:1.

          46. The method according to anyone of claims 30 to 33  
wherein the antiviral active compounds and the  
therapeutic agents are present in a ratio between  
25           about 1:50 to about 50:1.

          47. The method according to anyone of claims 30 to 33  
wherein the antiviral active compounds and the  
therapeutic agents are present in a ratio between  
30           about 1:20 to about 20:1.

          48. The method of claim 34 wherein antiviral active  
35           compounds and the therapeutic agents are present in a  
ratio between about 1:250 to about 250:1.

          49. The method of claim 34 wherein the antiviral  
40           active compounds and the therapeutic agents are  
present in a ratio between about 1:50 to about 50:1.

          50. The method of claim 34 wherein the antiviral  
45           active compounds and the therapeutic agents are  
present in a ratio between about 1:20 to about 20:1.

- 5 51. The method of claim 35 wherein antiviral active compounds and the therapeutic agents are present in a ratio between about 1:250 to about 250:1.
- 10 52. The method of claim 35 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:50 to about 50:1.
- 15 53. The method of claim 35 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:20 to about 20:1.
- 20 54. The method of claim 36 wherein antiviral active compounds and the therapeutic agents are present in a ratio between about 1:250 to about 250:1.
- 25 55. The method of claim 36 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:50 to about 50:1.
- 30 56. The method of claim 36 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:20 to about 20:1.
- 35 57. The method of claim 37 wherein antiviral active compounds and the therapeutic agents are present in a ratio between about 1:250 to about 250:1.
- 40 58. The method of claim 37 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:50 to about 50:1.
- 45 59. The method of claim 37 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:20 to about 20:1.

5 60. The method of claim 38 wherein antiviral active compounds and the therapeutic agents are present in a ratio between about 1:250 to about 250:1.

10 61. The method of claim 38 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:50 to about 50:1.

15 62. The method of claim 38 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:20 to about 20:1.

20 63. The use of at least one of (-)- $\beta$ -D-2,6-diaminopurine-1,3- dioxolane( $\beta$ -D-DAPD) and (-)- $\beta$ -D - 1,3-dioxolane guanine( $\beta$ -D-DXG) and at least one  
25 further therapeutic agent chosen from zidovudine, didanosine, zalcitabine, stavudine, lamivudine, nevirapine, delavirdine, efavirenz, indinavir, nelfinavir, saquinavir or ritonavir for the treatment  
30 of viral infections.

35 64. The use of claim 63 wherein the  $\beta$ -D-dioxolane is at least 97% free of the corresponding (+) enantiomer.

40 65. The use of claim 63 wherein at least one further therapeutic agent is chosen from zidovudine, lamivudine, nevirapine and combinations thereof.

45 66. The use of claim 64 wherein at least one further therapeutic agent is chosen from zidovudine, lamivudine, nevirapine and combinations thereof.

50 67. The use according to anyone of claims 63 to 66 wherein the viral infection is an HIV infection.

- 5           68. The use according to anyone of claims 63 to 66  
          wherein the compounds and the other therapeutic  
          agents are used sequentially.
- 10          69. The use according to claim 67 wherein the  
          compounds and the other therapeutic agents are used  
          sequentially.
- 15          70. The use according to anyone of claims 63 to 66  
          wherein the compounds and the other therapeutic  
          agents are used simultaneously.
- 20          71. The use according to claim 67 wherein the  
          compounds and the other therapeutic agents are used  
          simultaneously.
- 25          72. The use according to anyone of claims 63 to 66  
          wherein the compounds and the other therapeutic  
          agents are present in a synergistic ratio.
- 30          73. The use according to claim 67 wherein the  
          antiviral active compounds and the therapeutic agents  
          are present in a synergistic ratio.
- 35          74. The use according to anyone of claims 63 to 66  
          wherein antiviral active compounds and the  
          therapeutic agents are present in a ratio between  
40          about 1:250 to about 250:1.
- 45          75. The use according to anyone of claims 63 to 66  
          wherein the antiviral active compounds and the  
          therapeutic agents are present in a ratio between  
          about 1:50 to about 50:1.



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76. The use according to anyone of claims 63 to 66 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:20 to about 20:1.

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77. The use according claim 67 wherein antiviral active compounds and the therapeutic agents are present in a ratio between about 1:250 to about 250:1.

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78. The use according to claim 67 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:50 to about 50:1.

25

79. The use according to claim 67 wherein the antiviral active compounds and the therapeutic agents are present in a ratio between about 1:20 to about 20:1.

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80. The use of a pharmaceutical combination according to anyone of claims 1 to 29 for the manufacture of a medicament.

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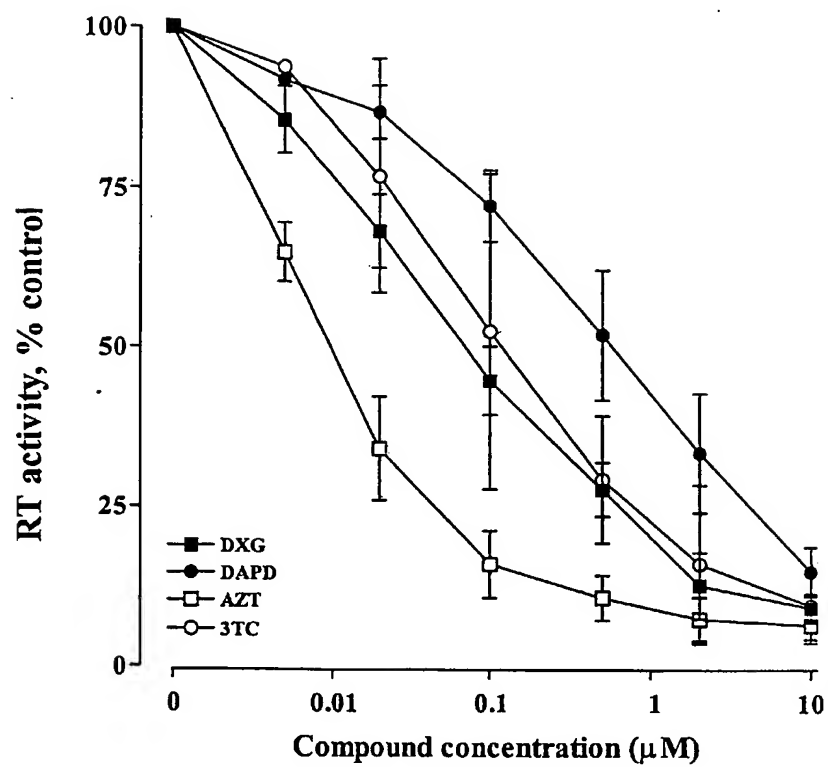


Fig. 1 Dose response curve of inhibition of HIV-1 replication.

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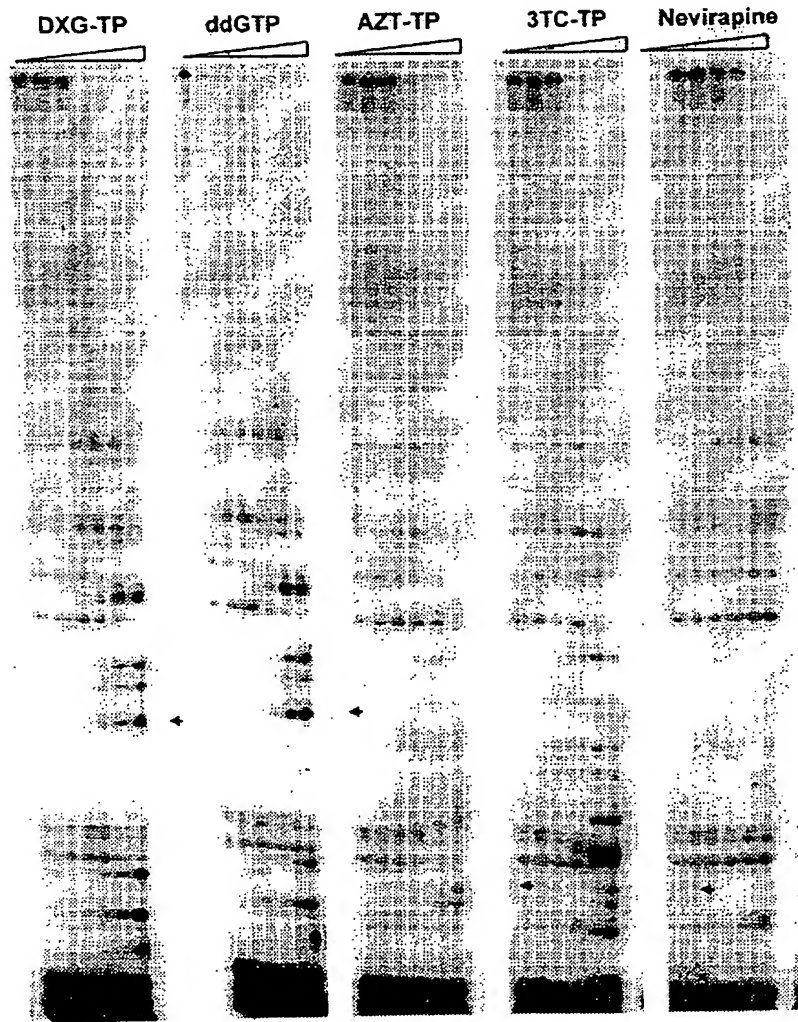


FIG. 2

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA 00/00212

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61K45/06 A61P31/12 A61P31/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GU ZHENGXIAN: "Mechanism of action and in vitro activity 1',3' dioxolanylpurine nucleoside analogues against sensitive and drug-resistant human immunodeficiency virus type 1 variants" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 43, no. 10, 1999, pages 2376-2382, XP000911148 page 2376 page 2379, column 2 page 2380, column 2 page 2381, column 1	1-6, 10-12, 15-23, 30-34, 37-40, 43-50, 57-67, 70-79
A	US 5 637 574 A (C.L.BURNS E.A.) 10 June 1997 (1997-06-10) claim 1 column 3, line 57 -column 4, line 16	1,5,7,8

☐ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

11 July 2000

Date of mailing of the international search report

19/07/2000

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 00/00212

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		ZA 9300041 A	05-07-1994

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